

The University of Chicago

CONTROL OF THE POTENTIAL RHYTHM
OF THE ISOLATED FROG BRAIN

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1939

By
BENJAMIN LIBET

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF NEUROPHYSIOLOGY
Vol. 2, No. 2, March 1939

The University of Chicago

CONTROL OF THE POTENTIAL RHYTHM OF THE ISOLATED FROG BRAIN

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1939

By
BENJAMIN LIBET

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF NEUROPHYSIOLOGY
Vol. 2, No. 2, March 1939



CONTROL OF THE POTENTIAL RHYTHM OF THE ISOLATED FROG BRAIN*

BENJAMIN LIBET

From the Department of Physiology, University of Chicago

(Received for publication January 3, 1939)

INTRODUCTION

THE PRESENCE of "spontaneous" rhythmic electric potentials in groups of nerve cells has been observed by most students of electrophysiology. These potentials exist in the absence of deliberately evoked nervous impulses (Berger, 1929; Bartley and Bishop, 1933; Gerard, Marshall and Saul, 1936; and others) and they persist, even for hours (Gerard and Young, 1937), in isolated groups of nerve cells (Adrian, 1931; Adrian and Buytendijk, 1931) despite the blocking of synaptic conduction by nicotine (Libet and Gerard, 1938). Such facts give impressive evidence that the rhythms are truly spontaneous, in the sense that no nerve impulses are immediately necessary to their evocation.

Several laboratories have investigated the physico-chemical factors influencing these rhythms in man and laboratory mammals (Dubner and Gerard, 1936 and 1939; Hoagland, 1936; Hoagland *et al.*, 1937; Lennox *et al.*, 1938; Jasper, 1936; Davis, 1936; see also Prosser, 1933, on the crayfish). The present experiments, directed to the same end, have been carried out under the much simpler conditions offered by the isolated frog brain. Information has also accumulated on the influence of incoming nerve impulses on cell potentials and concerning the mechanisms of synchronization of cell groups.

METHODS

Preparation. The brains of grass frogs (*R. pipiens* and *R. foxinus*) and bullfrogs (*R. catesbiana*) were used. Results were similar for all, freshly captured animals yielding the best preparations. Potentials from the brains of bullfrogs average perhaps twice the amplitude of those of the smaller frogs, although some of the latter have shown the greatest activity; and bullfrogs were used for experiments on the olfactory nerves because of their larger structures. The brain can be exposed and removed either from the separated head (decapitation without anesthesia) or from the intact etherized animal. Decapitation is accomplished by a single snip of a pair of scissors at the angles of the mouth, with the top blade as far caudalward as possible. The dorsal skin is reflected and, with the eye and muscles, trimmed off on one side. The skull is rapidly twisted outward with sturdy forceps from the open rear end forward, the brain gently lifted to one side, and the cranial nerves, including the olfactory, cut through. The freed brain is lifted with a small spatula or forceps and placed in Ringer's solution in a small glass container. The dura mater often slides off as the brain is lifted with forceps; if not it is carefully cut away. Pressure or tension, especially on the olfactory bulbs, must be carefully avoided. The original decapitation must be by shearing rather than crushing and the olfactory nerves severed (with fine scissors slipped forward under the brain) before lifting the brain. After some practice, this whole procedure requires less than 2 min. and the isolated preparation manifests potential rhythms almost without exception. This procedure is not applicable to the bullfrog, with tougher bony plates, and the brain is exposed in the usual manner in the etherized animal. It is then covered with cotton soaked in Ringer's solution and the frog allowed to

* A preliminary report of this work appeared in *Amer. J. Physiol.*, 1938, 123: 128.

blow off the ether for 1.5 to 2 hr.; after which time the dura is reflected, the brain sectioned in front of the optic lobes, the olfactory, optic and oculomotor nerves cut, and the fore-piece of brain removed to the container. Grass frog brains give like results when removed by decapitation or after etherization. Occasionally, recordings were made from the exposed intact brain *in situ* with the animal immobilized.

Electrodes. Ag-AgCl-wick electrodes (Adrian, 1936) were used. Silver tubes (0.3 mm. internal diameter, 15 mm. long), attached to solder wires for separate adjustment of each electrode, were held in a clamp with universal adjustment. A Ringer-soaked cotton thread, 0.2 mm. in diameter and projecting 1 to 2 mm. from the tube, served as wick. With this in place, a current of 1.0 mA. for 2 hr. deposited AgCl between tube and thread giving an electrode of some 10,000 Ω . resistance. During 3 or 4 weeks, although stored in Ringer's solution, resistance rises to 30,000 Ω , when the electrode is discarded. One wick was regularly placed on an olfactory bulb, the other 10 mm. lateral in Ringer's solution (or on bone). During the recording, the solution was sucked away so that only a film connected brain and indifferent electrode. It was found possible repeatedly to reset electrodes and to replace and remove solution with no significant change in the amplitude of recorded potentials; so that physical short-circuiting was constant and physiological injury absent. The

Table 1.

mM/liter	Frog plasma (Fenn)	Ringer used
K	2.5	1.9
Na	103.8	111.0
Mg	3.0	—
Ca	2.0	1.1
Cl	74.3	114.0
HCO ₃	25.4	—
Lactate	3.3	—
Phosphate	3.1	—
	215	228

electrode was lowered just until the fluid on the brain and wick coalesced, for pressure soon disrupted the slow rhythm. Grounding the indifferent lead had no effect. Fine platinum electrodes (0.3 mm.) were sometimes used, with one or both placed on or in the olfactory bulb. These yielded larger potentials but less regular and less constant ones, due to pressure injury, and were employed only for special purposes.

Recording. The push-pull, resistance-capacity coupled amplifiers, cathode ray oscillograph and crystograph were used according to standard practice in this laboratory. The usual controls of the electrical system, *e.g.*, with electrodes on dead brain, established the validity of observations on the living tissue.

Solutions. The ionic composition of Ringer's solution (Starling, 1936) differed somewhat from that of frog plasma (Fenn, 1936; Table 1). Since addition of carbonate or phosphate in the amounts present in plasma did not affect results, their absence is immaterial. Phosphate buffer (pH 7.4, 1/75 or 1/150 *M*) was also without influence and, therefore, usually omitted when not required. Magnesium, in the concentration normally present, does affect potentials (see below), and its absence from the Ringer's solution may have modified the findings; the higher ratio of K to Ca + Mg in our solution than in plasma perhaps contributing to the final disruption of the rhythm; but the saline solution as used would not have masked the action of the added ions since their effects increased with the concentration and were also reversible. Substances to be tested were added to the Ringer's solution without equivalent removal of ions; the slight increase in osmotic pressure so produced being well below that required to alter potentials. For pH values between 6.0 and 7.8, phosphate buffer was used; below 6.0, acetate; and above 7.8, borate (with NaCl replacing KCl in the buffer). The final pH was checked to 0.02 units with glass or hydrogen electrodes. No calcium precipitated, even after long standing of the buffers.

The solution surrounding the brain was changed by a standard procedure, to favor constant diffusion and other conditions of action of the substance tested. The electrodes

were raised, the solution sucked out, the brain and electrodes washed twice by gentle flooding with new solution and then left covered by it for 3 min.; finally the fluid was sucked out and the electrodes lowered into place. Replacing old Ringer's solution with fresh, to control the procedure, caused no change in potentials.

Temperature. For most experiments, room temperature, 22°C., was sufficiently constant. When studying temperature itself, the brain container was submerged almost to the rim in water in a Dewar flask and the brain nearly covered with saline at the same temperature. One junction of a thermocouple, lagged thermally with paraffin, hung into the water, the other was inserted into a cerebral hemisphere posterior to the bulbs. The water temperature was directly measured to 0.1°C. and did not change this much during a single run. Any difference between the temperature of the brain and the water was measured via the thermocouple to 0.05°C., a single determination requiring less than 10 sec. The actual temperature of the cerebral hemisphere was thus accurately followed as the system was changed from one temperature to another. Since electrical records were obtained from the olfactory bulb, with a wick electrode conducting heat, rather than from the hemisphere, where temperature was measured with 38-gauge wire conductor, other controls (dead brain, electrode and thermocouple together on bulb, etc.) were made to test the temperature comparability. No difference was seen in the rate of attaining temperature equilibrium by the two brain regions. The maximal temperature error possible in these measurements, 0.1°C., would not alter the estimated Q_{10} by 1 per cent.

Stimulation. Olfactory nerves were sometimes stimulated by induction shocks via platinum electrodes fixed in the container. The primary was fed waves at 40 to 60 per sec. from a thyratron. Stimulus artifact could not be eliminated but recording was started in less than 1 sec. after the end of tetanization.

RESULTS

Experiments on over 200 brains were directed to the study of such basic influences as temperature, salt and hydrogen ions, etc., on potentials. Such information is valuable in itself, and is also necessary to the examination of the action of more specific substances to be reported later.

Spontaneous rhythm

In vivo. The exposed uninjured olfactory bulbs of a frog, after recovery from anesthesia, exhibit a basic rhythm which is ordinarily constant in frequency and amplitude in any individual to within 5 per cent, irregularity being partly due to variation in superposed higher frequency waves. The fundamental frequency averages 6.0 per sec., extremes 4.0 and 8.5, with an average amplitude of 30 μ V., extremes 10 and 40. The whole surface of the bulb is electrically active. Exploration with a fine electrode (*in situ* and after isolation) shows the greatest and most regular potentials on the free lateral aspect and on the dorso-medial surface; the dorsal aspect between these is less active and the ventral still less. Electrodes on both bulbs record the same potentials as an active electrode on one and an indifferent electrode elsewhere, showing lack of electrical synchrony of the two sides. The faster feebler rhythms encountered in other parts of the brain (Gerard and Young, 1937) have not been further examined.

In vitro. Immediately after isolation of the brain, the olfactory bulb rhythm is much stronger, average 100 μ V., and more regular in height and wave form. Its average frequency is not altered but the moment-to-moment variability is decreased, from 5 per cent to 2 (Fig. 1A a and b). This powerful beat continues in the absence of measurable impulses from the olfactory

nerve or the remaining brain; for the former shows no action potentials and the electrical record from the hemisphere is flat in 15 to 20 min. after isolation, that from the optic lobe in 30 min. The bulb itself may continue to

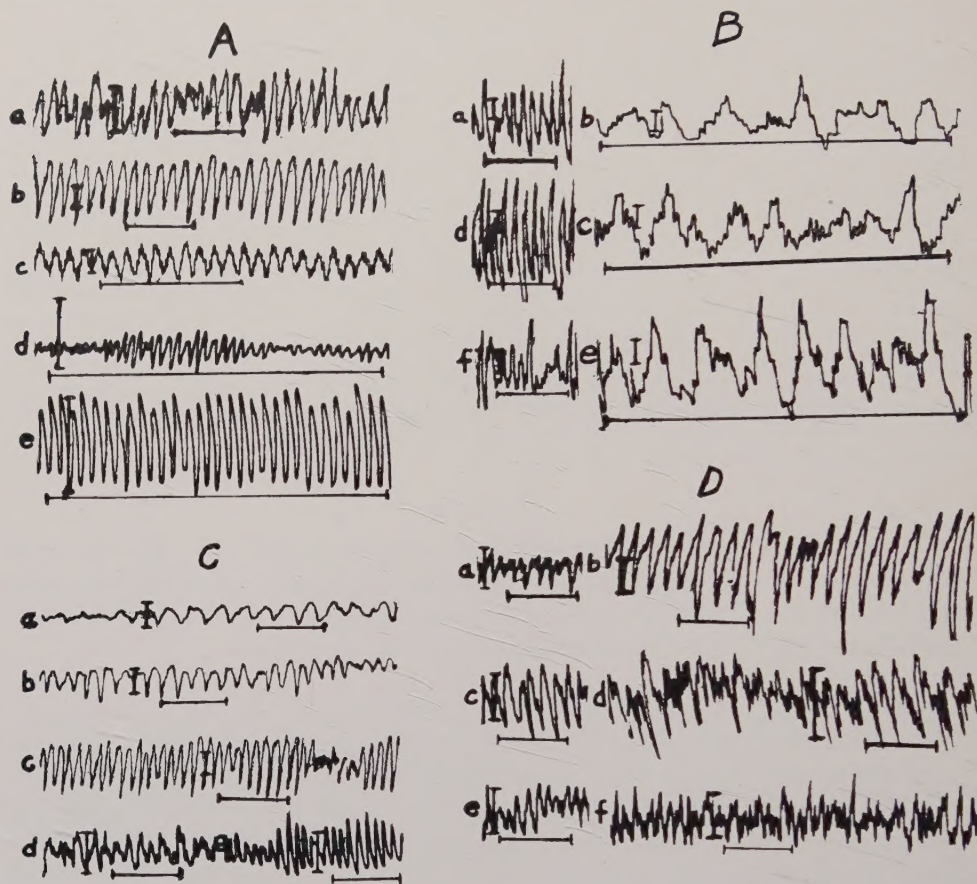


FIG. 1. A. a. Olfactory bulb *in situ*. b. Same bulb 1 min. after isolation of brain. c. Two bulbs separated from each other but attached to hemisphere. d. Single separated bulb. e. Single bulb fragment, about 0.1 mg.

B. a and b. Normal. c. 3 sec. after a 12-sec. stimulation of the olfactory nerves. d, e, f. 4.5, 6.5 and 13 min. after stimulation.

C. Bulb at: a. 15°C.; b. 22°C.; and c. 33°C. Another at: d. 22°C.; e. 28°C.

D. a, c, e, normal controls, each a separate brain; b, d, f. after 5 min. in test solutions. b. Ringer's plus glucose to double osmotic pressure. d. Ringer's plus acetate buffer, pH 5.5. f. Ringer's plus borate buffer, pH 9.0. Electrical records in all figures are from the olfactory bulb of an isolated brain except as indicated. The horizontal straight line represents one second, read left to right, the vertical one, 30 μ V, in all cases.

give potentials for 3 hr. or more, even with no special handling. Often after 10 to 15 min. the steady rhythm begins to wax and wane, and after 30 to 60 min. individual waves become somewhat irregular. Irregularity slowly increases and one or more faster rhythms (10 to 45 per sec.) come to supplant

the usual one. Amplitude falls with increased frequency and decreased regularity until the waves finally fade into the baseline. If frog serum is used in place of Ringer's solution, the 6 per sec. rhythm is retained longer (60–90 min.), irregularity is not so marked, and activity is not finally lost until some 4 hr. after isolation.

Further separation of the bulbs, by cutting away the hemispheres with a razor, results in inactivity, due to pressure injury by the blade. Such pressure was avoided by separating the brain tissue with two pairs of finely ground forceps, the tissue not immediately about the instrument thus being spared mechanical insult. In similar fashion, the two bulbs were later separated from each other, and even a single bulb picked to pieces. Each successive step led, in the majority of cases, to a discontinuous increase in frequency. Thus, for example, removing the hemispheres raised the frequency from 6 to 8 per sec.; separating the two bulbs raised it to 10 per sec.; isolating a bulb completely, to 30 to 40 per sec.; and, finally, isolated bulb fragments of about 0.1 mg. gave a regular rhythm, *e.g.*, at 30 per sec. and of 15 μ V. intensity (Fig. 1A c, d, e). There was usually a corresponding loss in amplitude, but occasionally an increase occurred in this as well as in the frequency. In two instances, bulbs which had ceased activity regained it after separation from the hemispheres; and, in another case, activity was enhanced at once on separation, and continued to increase further during several minutes.

Effects of stimulation

The return or increase of olfactory bulb potentials produced by isolation procedures must be due to removal of inhibitory control or to direct excitation. The latter could be further tested by electrical stimulation of the olfactory nerves or the bulbs themselves. Tetanizing bulbs which had ceased activity restored spontaneous potentials in 3 of 5 cases. The potentials were feeble and irregular and resembled those usually seen some 20 min. prior to inactivity. When the nerves were stimulated (10 sec. 40 shocks per sec.) in the freshly isolated brain, the regular rhythm was disrupted and replaced by irregular small 30 to 50 per sec. waves. This picture outlasted the stimulation for 2 to 3 sec., when the 6 per sec. rhythm reappeared, to increase in amplitude and regularity during another 2 to 4 min. (Fig. 1B); by this time *i.e.*, 4 to 7 min. after stimulation, the regular rhythm had attained an amplitude 25 per cent greater than before stimulation and the intensity then fell slowly, over an average period of 12 min., until it became normal. More prolonged stimulation caused more enduring post-stimulation changes. In only 2 of 12 experiments was tetanization of the nerve without effect, synaptic transmission perhaps having failed in the 2 cases before the test.

Temperature

The temperature of the isolated brain was varied between 8 and 35°C. but usually only between 14 and 30°C. At lower temperatures potentials gradually flattened to basal level; at higher temperatures, wave amplitude,

after brief but conspicuous increase, fell abruptly and irreversibly. In moderate temperature ranges, amplitude and especially frequency of the rhythm increased with temperature (Fig. 1C). When the temperature was brought to a new level the brain attained the new temperature, as shown by a thermocouple, in 4 to 5 min. The rhythm change lagged behind this and might require 4.5 to 6.5 min. to reach a new steady value (Table 2). In 16

Table 2. Exp. 3/8/38. Bullfrog. Ether at 2:00 p.m. Brain exposed 2:15 p.m., isolated (to middle of cerebral hemisphere) 4:35 p.m. Readings start at 4:50 p.m.

Temp. (Centigrade) around brain	Time (min.)	Brain temp.	Frequency (per sec.)	Q ₁₀ of frequency	Ampli- tude (μV.)	Q ₁₀ of amplitude
23° to 29°	0 to 0.25 1.0 1.75 2.5 3.25 4.0 4.5	22.9 24.4 26.0 26.4 26.9 26.9	7.0 7.8 9.0 9.5 10.1 10.1	$\left. \begin{array}{c} 2.2 \\ 2.5 \\ 3.6 \end{array} \right\}$	60 70 75 80 75	$\left. \begin{array}{c} 1.9 \end{array} \right\}$
to 23.5°	to 4.75 6.0 7.6 16.0	 24.3 23.6 23.2	 7.8 7.5 6.0	$\left. \begin{array}{c} 2.7 \\ 2.5 \end{array} \right\}$	 60 65 70	$\left. \begin{array}{c} 1.5 \end{array} \right\}$
* to 16°	 to 16.25 17.0 17.3 18.25 19.4 20.65 23.1	 20.2 19.0 17.7 17.0 16.5 16.2	 5.5 5.1 4.0 3.6 3.4 3.0	$\left. \begin{array}{c} 1.5 \\ 2.1 \\ 5.7 \end{array} \right\}$	 65 55 55 45 40 40	$\left. \begin{array}{c} 2.1 \end{array} \right\}$
to 23.3°	to 23.75 25.5 27.75	 21.5 22.7	 6.0 6.3	$\left. \begin{array}{c} 3.7 \\ 3.0 \end{array} \right\}$	 80 80	$\left. \begin{array}{c} 2.7 \end{array} \right\}$

* Q₁₀ between 26.9°-16.2° is 3.1, but this high value must be discounted because of a break in the experiment at this point.

experiments in the moderate temperature range, the Q₁₀ of frequency fell between 2.1 and 2.6, with an average of 2.3. The Q₁₀ for amplitude, although of the same order, was more variable. This was due to greater dependence of amplitude than of frequency on the time of exposure to each temperature and to "spindling" of wave trains, which necessitated the arbitrary use of half the maximal amplitude for the calculations. A typical experiment is shown in Table 2, and four are plotted in Fig. 2 where the Q₁₀ is calculated from Belehrádek's (1935) formula:

$$Q_{10} = \left(\frac{K_1}{K_2} \right)^{10/t_1-t_2}$$

where K_1 and K_2 are the wave magnitudes at the Centigrade temperatures, t_1 and t_2 , respectively. The Q_{10} is greater in the lower temperature range (cf. Gasser, 1931), and the coefficient tends to be greater for a given temperature step on passing from the lower to the higher value than vice versa. Besides thermal control of frequency and amplitude, the regularity of the waves consistently improves, with a rise in temperature, while cooling al-

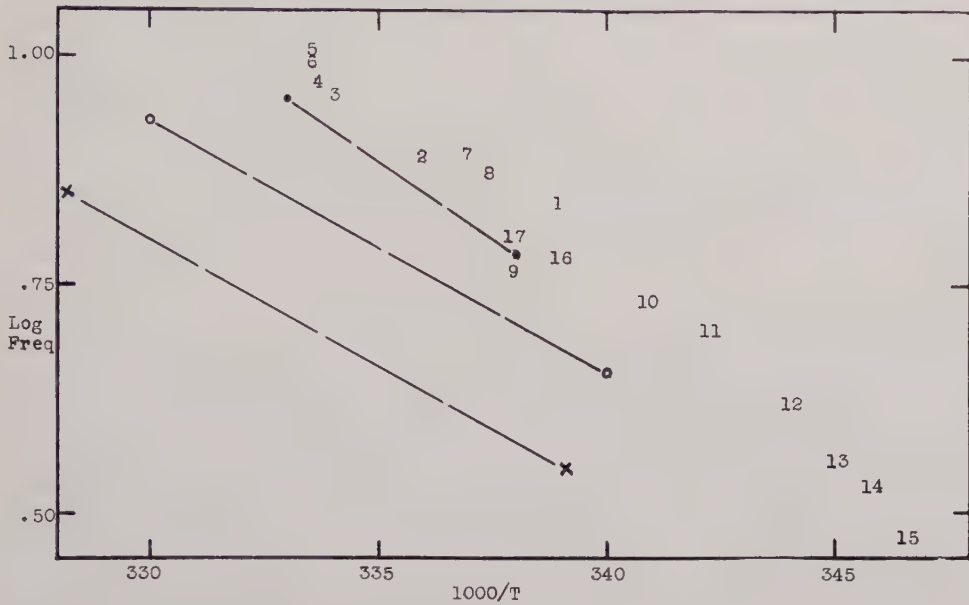


FIG. 2. Log. frequency plotted against reciprocal of absolute temperature. X, 0 and • data from 3 separate brains. The numerals are the observations of Table 2, the number giving the order in which observations were made, plotted to these coordinates.

ways makes the wave shape more irregular (Fig. 1C). Greater regularity means a better alignment of activity in time, regardless of what components make up the wave. Consequently the process of synchronization is facilitated by a rise in temperature.

Osmotic pressure

Addition of sucrose or glucose to the Ringer's solution, sufficient to increase osmotic pressure by 50 per cent, does not affect potentials. Doubling the osmotic pressure with either sugar caused a change after 3 min. soaking in 4 of 9 experiments. The waves slowed 15 to 20 per cent, *e.g.*, from 6.1 to 4.9 per sec., increased in regularity, and became 10 to 20 per cent larger (Fig. 1D a, b). The same osmotic change produced by doubling the Ringer salts, in 3 experiments, caused marked irregularity with the appearance of frequencies of 10 to 40 per sec. and a great loss of amplitude, not due solely to increased conductivity. Diluting Ringer's solution with an equal volume of water lowered the normal frequency only 10 per cent and made

the waves rather more regular; their amplitude was increased by half, after correcting for resistance changes. In water, potentials soon became irregular and disappeared after about 15 min. Most of the effects resulting from altered salt concentration depend more on specific ion changes than on changes in osmotic pressure.

H-ion concentration

Soaking in 0.013 *M* phosphate buffer has no effect on potentials. At pH values below 6.2 or above 7.8 changes are seen after 5 min. soaking. In acid solutions (acetate buffer) the 6 per sec. rhythm slows discontinuously to about 4 per sec. and the waves become less regular, with superposed frequencies of 15 to 40 per sec. (Fig. 1D c, d). In alkaline solutions (borate buffer), the normal rhythm became irregular and fairly strong waves at 15 to 30 per sec. are added (Fig. 1D e, f).

Electrolytes

The brain soaked in isosmolar (0.24 *M*) sucrose solution instead of Ringer's showed a progressive slowing of its rhythm and finally failed in 20 to 30 min. (Fig. 3A a, b, c, d, e). After 3 to 4 min. soaking, the 6 per sec. waves slowed sharply to 4 per sec. and increased 50 per cent in amplitude (e.g., from 120 to 180 μ V.). After another 5 min., frequency fell to 3 per sec., and an increased inter-electrode resistance indicated fairly complete loss of salts from the brain's conducting fluids. After 12 to 15 min. total soaking, the frequency again dropped sharply, to 1 per sec., irregular waves at 15 to 20 per sec. were often superposed, and the amplitude became less. The negative limb of these slow waves was longer than the positive and the high-frequency discharges occurred on the slight plateau of greatest negativity. Further soaking usually caused gradual loss of these potentials. In one case (with K and Ca still present; see below) the 1 per sec. waves were again supplanted by extremely regular rhythms at 4 per sec. Diphasic spike-like potentials of 200 μ V. interrupted this regular sequence, occurring first in groups of 3, then in mixed triple and single groups, then singly, and finally disappearing (Fig. 3B f, g, h). The diphasic spikes are probably produced by traveling rather than stationary potential waves.

These changes were only partially reversible. Soaking the brain in Ringer's solution, after activity had disappeared in the non-electrolyte solution, led to feeble irregular potentials. Restoration to saline at the slow wave stage replaced these with faster less intense ones which lack the normal regularity. A solution containing sucrose in place of NaCl, but with the other ions of Ringer's solution present in normal amounts, affected potentials as does pure sucrose, except that about twice as much time was required for each change and the slow waves did not become so extremely regular (Fig. 3B). Increasing the K-ion (to 0.004 *M*) partially antagonized the effects of sodium lack, and led to potentials much like those in Ringer's solution following sucrose (Fig. 3A f, g, h).

Sodium lack was mainly responsible for the changes seen in non-electrolyte solutions. Addition to or subtraction from Ringer's solution of NaCl

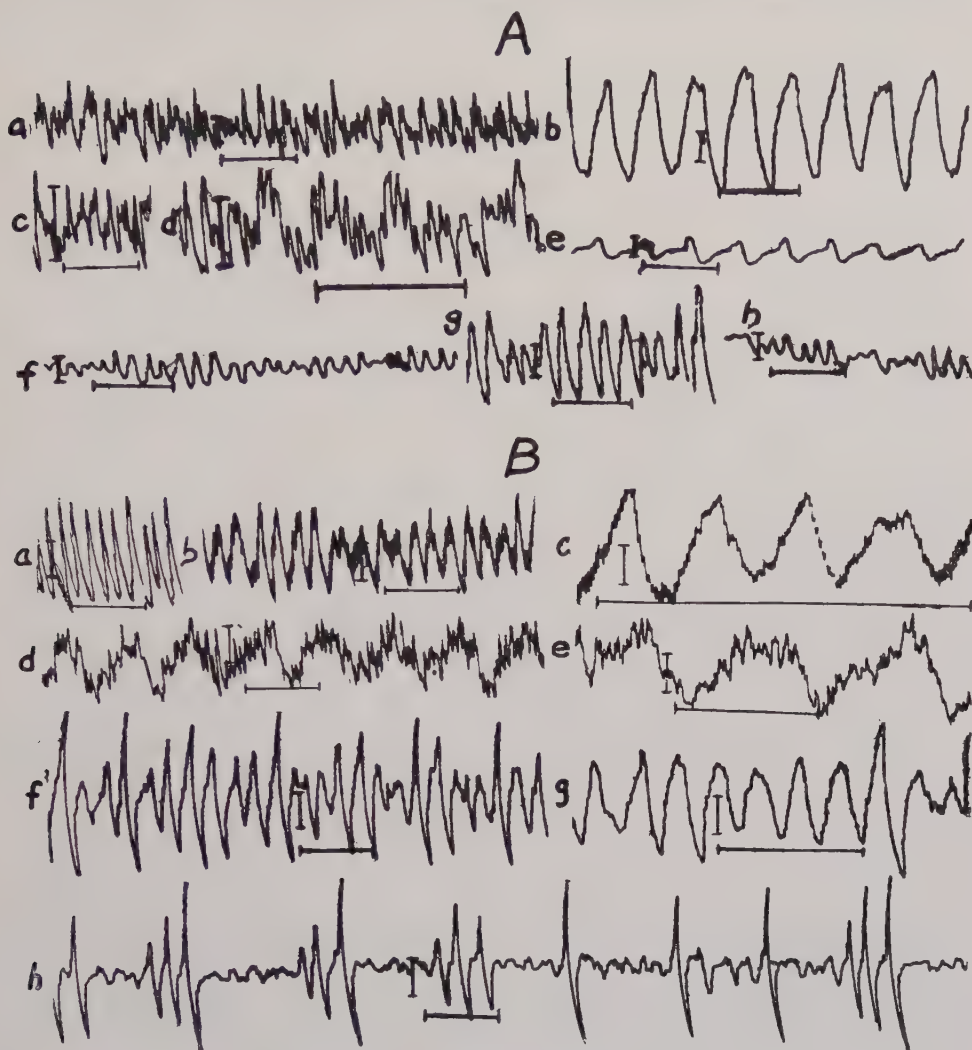


FIG. 3. A. a. Normal in Ringer. b. after 4 min. in 0.25 M sucrose. c. and d. 3 min. after return to Ringer. e. 8 min. after replacing in sucrose. f. Another brain in Ringer's after 6 min. in sucrose. g. 2 min. after return to sucrose. h. 3 min. after replacing sucrose with sucrose plus normal CaCl_2 and double KCl.

B. a. Normal in Ringer. b. and c. after 10 min. in Ringer with sucrose replacing NaCl. d. and e. after 20 min. in same solution. f. and g. after 30 min. h. 20 sec. after g.

in moderate concentration, e.g., 0.02 M, did not significantly affect potentials. Doubling Na-ion produced feeble irregular rapid potentials, though the increased osmotic pressure, *per se*, would act in the opposite direction. Replacing half the Na-ion of Ringer's solution with the isosmotic equivalent

of sucrose slowed the waves about 20 per cent after 3 min. soaking, 30 per cent after 10 min., increased their amplitude 30 to 40 per cent (e.g., from 110 to 150 μ V.), and increased regularity.

Potassium acted much like sodium, but much smaller quantities were effective. Isosmotic (0.125 *M*) KCl initiated rapid feeble waves which faded completely after soaking for 4 min. In 0.01 *M* concentration, this salt led to

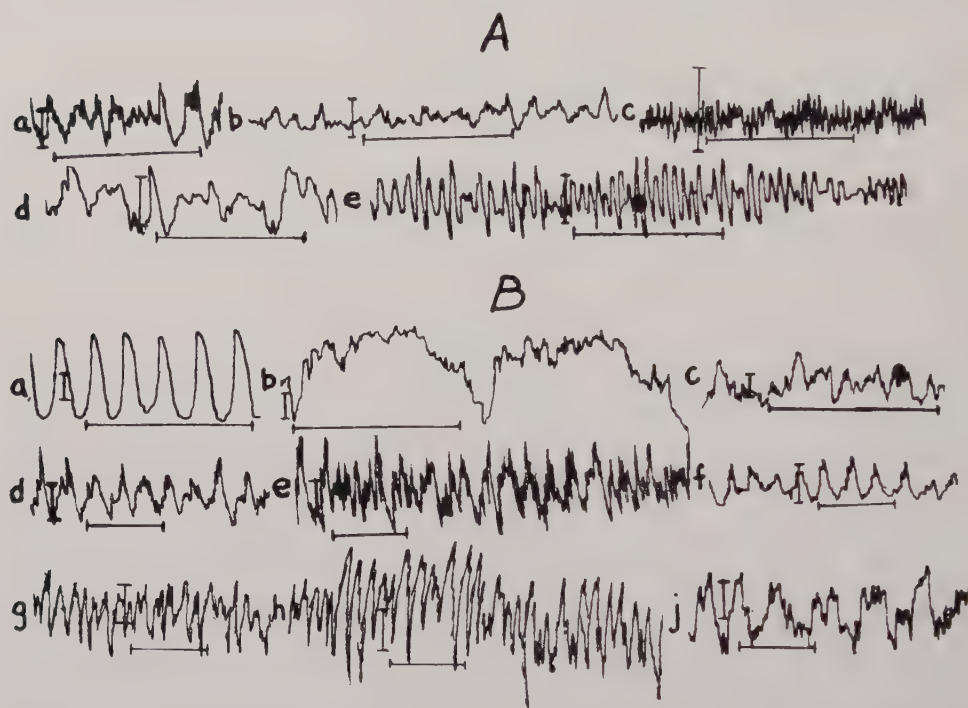


FIG. 4. A. a. normal in Ringer. b. after 3 min. in Ringer plus 0.003 *M* KCl. c. after 3 min. in Ringer plus 0.01 *M* KCl. d. another brain, normal. e. after 3 min. in Ringer plus 0.01 *M* KCl.

B. a. normal in Ringer. b. after 3 min. in Ringer plus 0.018 CaCl_2 . c. 3 min. after return to Ringer. d. another brain, in Ringer plus 0.003 *M* CaCl_2 . e. 3 min. after placing in Ringer plus 0.005 *M* Na citrate. f. 3 min. after return to Ringer plus 0.003 *M* CaCl_2 . g. another brain, normal in Ringer. h. after 3 min. in Ringer plus 0.003 *M* MgCl_2 . j. after 3 min. in Ringer plus 0.01 *M* MgCl_2 .

irregular potentials in some cases, to regular waves at 20 to 30 per sec. in others (Fig. 4A). Concentrations between 0.003 and 0.01 *M* caused a sharp change from the normal to feeble somewhat irregular waves at 15 to 35 per sec. These changes were but little reversible when the brain was restored to Ringer. Weaker KCl, 0.002 *M*, however, gave a largely reversible increase in rate and decrease of amplitude (50 per cent) and regularity.

Calcium and magnesium (Fig. 4B) acted alike, and at similar concentrations, to change potentials in a manner opposite to that of sodium and potassium. Addition of 0.001 *M* CaCl_2 to Ringer's solution definitely slowed

the waves, and the effect was progressive with concentration to 0.007 *M*, which lowered frequency by 35 to 45 per cent (*e.g.*, from 7 to 4 per sec.). Amplitude was not altered and regularity perhaps improved. Larger Ca-ion additions, 0.018 *M*, caused a discontinuous further slowing to 1 per sec. waves of the same amplitude with feeble irregular potentials at 10 to 20 per sec. superposed. Return to Ringer's solution restored more rapid waves, but they never regained the normal regularity. Removal of calcium affected potentials as did addition of potassium. Sodium citrate (0.004 *M*) in Ringer's solution produced irregular rapid waves, and the normal slow rhythm was again restored by Ringer's solution with added CaCl_2 (0.003 *M*) (Fig. 4B d, e, f). Sodium sulphate acted like citrate but greatly depressed amplitude as well (*e.g.*, from 35 to 5 μV), and its action was irreversible.

Ion antagonism was apparent from the above results and clearly showed when potassium and calcium, especially, were balanced against one another. When both were removed from Ringer's solution, potentials in the remaining NaCl continued quite normal for about 10 min.; when both were added in equimolar amounts, up to at least 0.005 *M* addition, potentials also continued normal for some time; and when the excess of either ion had changed potentials, a slight imbalance in favor of the other improved and hastened the restoration toward normal.

Anions had little effect on potentials, except as they removed calcium. (Sulphate probably has a specific action as well.) At pH 7.4, mono- or dihydrogen phosphate (up to 0.013 *M* concentration) and bicarbonate (up to 0.02 *M*) were inactive. Substitution of half the chloride ion in Ringer's solution by iodide ion was without effect, and complete interchange of the two produced only minor differences. (Peculiar diphasic waves appeared at intervals in iodide.)

DISCUSSION

A single olfactory bulb can be made to vary its regular rhythm over a wide frequency range by appropriate change in environmental conditions. All rates have been encountered between 1 and 10 per sec., the values 13 and 17 appear and, less sharply, rates of 20 to 25, 35 to 40, and 45 to 55 (Fig. 5). Amplitude tends to decrease with increasing frequency, as does regularity, but large or small waves have been seen at all rates. Further, the shape of the individual regularly repeated waves can be deliberately changed from an approximate sine form to a variety of highly unsymmetric profiles (Fig. 5).

The relatively homogeneous cells of the bulb, probably only a few thousand separate units altogether, can thus manifest potentials of a fixed regularity and even a fixed amplitude over a fifty-fold range in frequency and a wide array of wave shapes. These facts speak strongly for the existence of individual neurone potentials of frequency and form identical with those of the recorded potential. This latter is the integral of the unit potentials and will reproduce them, except in amplitude, only if all units are similar and in synchrony. With asynchrony of units no regular pattern could be recorded unless each cell became active in a constantly repeated time se-

quence, as in the case of the flashing lights of a sign which are engaged in order by a rotating contact drum. The reverberating circuits postulated for the brain (Lorente de Nô, 1938) might afford just the neural mechanism required for such a delicate timing; but they are unfortunately ruled out by experiments with nicotine (Libet and Gerard, 1938; Schweitzer and Wright,

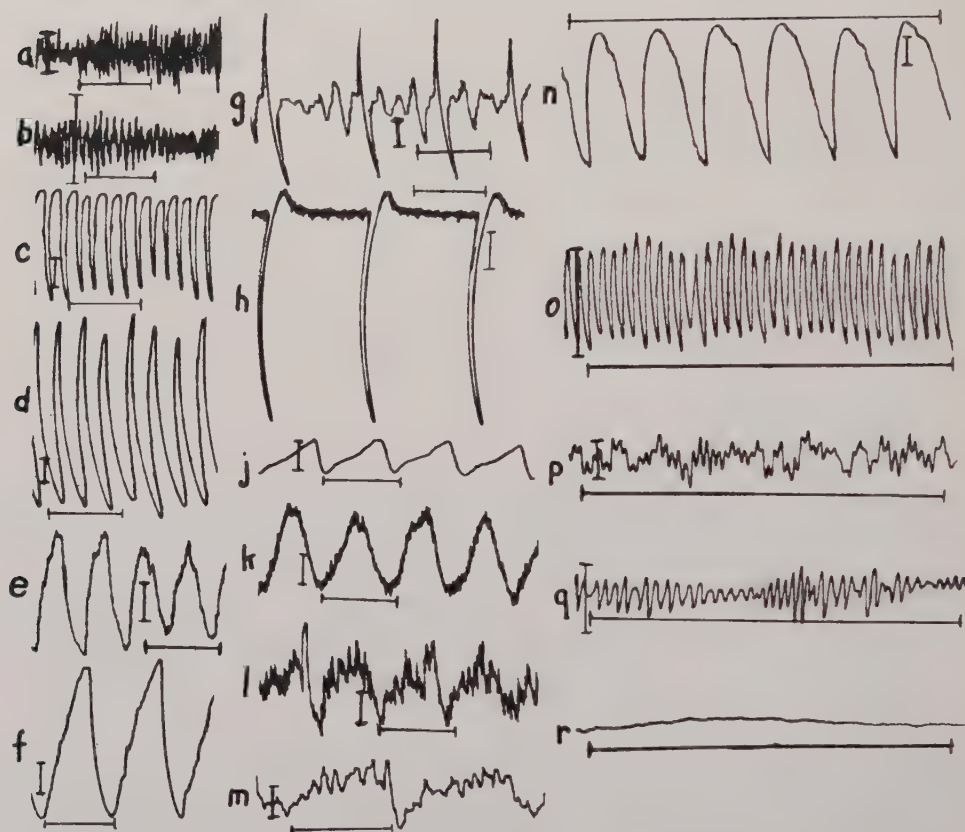


FIG. 5. Samples of rhythms obtained from isolated olfactory bulbs. a. freshly isolated, aberrant. b. 2 hr. in serum. c. freshly isolated. d. 3 min. in nicotine. e. 3 min. in sucrose. f. 6 min. in nicotine. g. 30 min. in Ringer with sucrose in place of NaCl. h. 3 hr. in stale serum. j. 10 min. in nicotine. k. 4 min. in nicotine. l. 15 min. in Ringer with sucrose in place of NaCl. m. 20 min. in Ringer with sucrose in place of NaCl. n. freshly isolated. o. small isolated bit of bulb. p. 30 min. in Ringer, aberrant. q. single separated bulb. r. control on optic lobe.

1938), which blocks synaptic conduction but leaves perfectly regular odd-shaped waves.

The range of frequencies exhibited by cells of one or few anatomical types (Gerard and Young, 1937; Adrian, 1937) must, then, represent a range of frequencies for each unit (see Blake and Gerard, 1937) or a regular sequence of action of varying numbers of cell groups. To the extent that doubling of frequency is accompanied by halving of amplitude, such an

alternation would be possible. But, though this is common, there are plenty of instances of a frequency increase with constant amplitude. Further, frequency may change smoothly with no discontinuities, or with jumps to values that are far from simple multiples of the initial ones. And, finally, any such beating in fractional groups would not account for a change to skew wave-forms, as produced by sucrose, nicotine, etc. The simplest hypothesis again seems to be that many single units beat in synchrony and so give a magnified record of their individual identical frequencies. This also fits with the fact that regular waves are obtainable from small fragments of teased olfactory bulb. It may be also, as Adrian (1937) suggests, that synchronizing occurs best at certain cell frequencies.

Changes in rate and form and, within limits, in amplitude induced by applied agents in *regular* potential waves would, then, be interpreted in terms of the individual neurone beat. But changes in regularity also occur—from an increased variability of successive distinct waves, through “fuzzy” waves with superposed potentials, and irregular alternations, to highly random potential swings—and these are best interpreted in terms of the mechanism of synchronization. Slight cell asynchrony will cause more apparent irregularity in short fast waves than in long slow ones; yet increase in temperature, which accelerates the rhythm, also improves its regularity. This is an especially clear case for a synchronizing mechanism, independent of the individual cell, which increases in efficiency with temperature. We shall, therefore, interpret the action of experimental procedures on potentials in terms of the unit neurone beat and the degree of synchronization of units.

Each cell exhibits a potential wave with amplitude, wave-length and shape characteristic for the extant conditions. The relation between frequency and intensity is especially important since, by physical analogy, at constant energy (from cell metabolism) the two should vary inversely. A change of rate without a change in amplitude, still more a parallel increase or decrease in both together—as in the case of altered temperature—must, then, indicate a change in available metabolic energy. (With improving synchrony, of course, amplitude could increase in the total record and not in the unit. This can often be excluded.) On the other hand, increased frequency with decreased amplitude might represent a change in the “setting” of some “trip” mechanism, with no change in the original metabolic sequences. The first case above would correspond to a change in the tension of a metronome spring, the second to a shift in position of the ballast on the pointer. Frequency variation due to alterations in metabolic rate would almost surely be smoothly continuous and essentially reversible, as with temperature; but variation due to resetting of the trip mechanism (some membrane property?) might easily be discontinuous and imperfectly reversible, as with altered ionic environment. Many agents unquestionably have a mixed action; and we shall report in a later paper studies, of the influence on these potentials of a number of metabolic inhibitors, accelerators, and other effective drugs, that help to clarify the cellular mechanisms which determine the electrical beat.

Little can be said as yet about the mechanisms which integrate and synchronize the beats of individual cells, but that such exist is certain. The findings with nicotine, etc., show that, though the passage of nerve impulses along conducting paths may play an important role, this is not a necessary or indeed the most important means of coordination. It has been suggested (Gerard, Marshall and Saul, 1936) that steady potential fields and polarization might contribute to synchronization, and a small constant current does increase cat brain rhythms as if improving cell unison (Dubner and Gerard, 1939). This experiment has not yet been performed on the frog brain, but some similar evidence is available from waxing and waning of waves.

Regular wave trains do not wax and wane but as the single waves become less constant this spindling is likely to appear. An interpretation would be that the cells are locked together by a strong synchronizing mechanism to give regular beats and that as this control is lessened the cells are more easily desynchronized, with first some fuzziness in the recorded potentials and then the coming in and out of phase which produces amplitude beats. There are indications that a steady potential of the brain mass is more negative during the large waves at the belly of a spindle and less so when the feeble waves occur at the ends of one. A greater constant potential is, therefore, associated with greater cell unison. That regular waves document a strong action of the synchronizing mechanism is further shown by attempts to disrupt them. It is constantly found that an agent which disturbs a steady rhythm, for example K ion, is effective in lower intensity with less regular waves. A very smooth beat persists in KCl concentrations which break up a less constant one and is disrupted only by greater concentrations.

These effects are seen with the passage of time *in vitro* and it is likely that one factor in the disintegration of the potentials of an isolated brain is the gradual failure of the synchronizing mechanism. The effects of stimulation might be partly via an improvement of this; and the fact that further isolation of a small bit of brain from a larger inactive mass brings out again a potential beat could also be explained in terms of leading from a few synchronized units rather than from many desynchronized ones. Finally, the improved synchrony induced by rise in temperature and by certain stimuli and by nicotine, sucrose or calcium, and the diminished synchrony with time of isolation, cold, potassium, etc., show that the synchronizing mechanism is a real entity with measurable properties permitting its further analysis.

It remains to note some impressive parallels between the behavior of brain and nerve. Brain rhythms have been compared to the oscillating after-potentials of nerve (Gerard, 1936; Gasser, 1937). In mammalian nerve (Lehmann, 1937a and 1937b; less completely in frog nerve, Graham, 1933) the after-potential rhythm is increased and the "wave-length" shortened by raised potassium or by lowered calcium or hydrogen ion. The reverse ion changes, although augmenting the first negative after-potential, lengthen the oscillation period and may completely depress the waves. These effects are entirely comparable to those produced in the frog's olfactory bulb (and

cat's thalamus (Dubner and Gerard, 1939), although the ions act on the brain more rapidly and in lower concentration. (Compare Dusser de Bar-enne, McCulloch, and Nims, 1937, on the influence of pH on cortical potentials.) That the frog brain should be relatively insensitive to pH changes is not surprising in view of the fact that the pH of the frog's blood normally varies rather widely (see Rohde, 1920); also frog nerve metabolism is fairly insensitive to pH change (Gerard, 1930). With after-negativity of nerve goes heightened irritability and even spontaneous discharge (Gasser and Grund-fest, 1936; Lehmann, 1937a); with positivity, depression. The same is true for grey tissue (Adrian, 1931 and 1937; Hughes and Gasser, 1934; Eccles, 1936; Heinbecker, 1936; Barron and Matthews, 1938); and, in both, potassium and calcium powerfully control potentials, activity, and after-discharge (Gerard and Magoun, 1936). Further, in both brain and nerve there are similar powerful cation antagonisms while anions are relatively unimportant. Spike height in nerve is reduced by excess of either potassium or calcium (Graham, 1933) and the same is true in brain cortex (Dubner and Gerard, 1939).

Finally, nerve after-potentials are minimal in the well-rested tissue and increase with repeated activity. Their persistence, amplitude and oscillations, following a single impulse, depend on previous excitation and contemporary physico-chemical environment. The brain potential oscillations similarly are influenced by past activity as well as current environment; stimulation enhances the wave for a considerable time afterward and the usual rhythm fades out over hours in the unstimulated isolated bulb but can be restored for many minutes by a brief barrage of impulses. A like interpretation seems to fit the sequence of potential changes of the human cortex in sleep (Blake and Gerard, 1937; Blake *et al.*, 1939). It is also not improbable that the increased frequency commonly observed when a bit of brain is separated from a larger piece is due to injury potentials at torn processes, etc. It will be desirable to compare the behavior of such brain rhythms with that of the demarcation potentials of nerve and brain with change in time, temperature, ion concentration and the like.

The action of ions on brain and nerve metabolism has been reviewed elsewhere (Gerard, 1932 and 1937) and here it need only be mentioned that for brain respiration, as for its potentials, sodium and potassium synergize in increasing it, with potassium far more active per mole, and calcium and magnesium depress it; while anions have little effect (*e.g.*, Dickens and Greville, 1935). Whether the persistence of a rhythm in a salt-free medium means that inorganic ions are not indispensable to the potential control or that these are incompletely removed even from the cell exterior, cannot be answered from these results. At least the brain neurones have a sufficient store of substrate, and obtain adequate oxygen for the oxidative portion of their metabolism, to maintain their beat in isolation. Their ultimate failure is due only in part to loss of background excitation and must finally depend

on depletion of reserves or on disintegration of organization in the abnormal *in vitro* environment.

SUMMARY

The olfactory bulb of the isolated frog brain, which continues its *in vivo* electrical activity (especially a large regular 6 per sec. rhythm), was used to investigate physico-chemical and nervous factors controlling such spontaneous potentials.

Wave size and regularity are enhanced immediately upon isolation, without change in frequency, then gradually decrease to zero during about 3 hr. in Ringer's solution and 4 in serum. Wave frequency usually increases with each step of further isolation (single bulb, bit of bulb), and regularity, if anything, is improved.

Electrical stimulation can restore some activity of the "run down" brain; and, even in the freshly isolated one, stimulation of the olfactory nerve for seconds increases bulb potentials by 25 per cent for 10 min. following.

Rise in temperature, between 5 and 30°C., increases frequency (the average Q_{10} is 2.3) and amplitude, and always improves regularity. Cooling has the reverse effect.

Doubled osmotic pressure, radically reduced Na-ion, moderately increased Ca- or Mg-ions, or lowered pH produce slow waves; while increased K, Na, or pH and reduced Ca produce fast ones. Na and K are antagonistic to Ca, K in small concentrations being more effective than Na. Effects are generally progressive with concentration. Changes in frequency are usually discontinuous, and when extreme are irreversible. Anions are generally without marked effects.

Although originating in a small homogeneous neurone population, potential patterns may vary greatly in frequency, wave shape, and regularity depending on the factors studied. These and other related facts are discussed in relation to the problems of frequency and amplitude of single neurone rhythms and of the mechanism coordinating them to give the recorded potential. Significant parallels appear between rhythmic nerve potentials and those of cerebral neurones.

REFERENCES

- ADRIAN, E. D. Potential changes in the isolated nervous system of *Dytiscus marginalis*. *J. Physiol.*, 1931, 72: 132-150.
- ADRIAN, E. D. The spread of activity in the cerebral cortex. *J. Physiol.*, 1936, 88: 127-161.
- ADRIAN, E. D. Synchronized reactions in the optic ganglion of *Dytiscus*. *J. Physiol.*, 1937, 91: 66-89.
- ADRIAN, E. D., and BUYTENDIJK, F. J. J. Potential changes in the isolated brain stem of goldfish. *J. Physiol.*, 1931, 71: 121-135.
- BARRON, D. H., and MATTHEWS, B. H. C. Electrotonus in ventral roots of spinal cord. *J. Physiol.*, 1936, 87: 26 P.
- BARTLEY, S. H., and BISHOP, G. H. The cortical response to stimulation of the optic nerve in the rabbit. *Amer. J. Physiol.*, 1933, 103: 159-172.
- BERGER, H. Über das Elektroenkephalogramm des Menschen. *Arch. Psychiat. Nervenkr.*, 1929, 87: 527-570.
- BELEHRÁDEK, J. Temperature and living matter. *Protoplasma Monographien*, 1935, 8, Berlin, Gebrüder Borntraeger, x, 227 pp.
- BLAKE, H., and GERARD, R. W. Brain potentials during sleep. *Amer. J. Physiol.*, 1937, 119: 692-703.

- BLAKE, H., GERARD, R. W., and KLEITMAN, N. Factors influencing brain potentials during sleep. *J. Neurophysiol.*, 1939, 2: 48-60.
- DAVIS, H. Some aspects of the electrical activity of the cerebral cortex. *Cold Spr. Harb. Symp. quant. Biol.*, 1936, 4: 285-292.
- DICKENS, F., and GREVILLE, G. D. The metabolism of normal and tumour tissue, XIII. Neutral salt effects. *Biochem. J.*, 1935, 29, P. 1: 1468-1483.
- DUBNER, H., and GERARD, R. W. Factors controlling brain potentials in the cat. *J. Neurophysiol.*, 1939, 2: 142-152.
- DUSSER de BARENNE, J. G., McCULLOCH, W. S. and NIMS, L. F. Functional activity and pH of cerebral cortex. *J. cell comp. Physiol.*, 1937, 10: 277-291.
- ECCLES, J. C. Synaptic and neuromuscular transmission. *Ergebn. Physiol.*, 1936, 38: 339-444.
- FENN, W. O. Electrolytes in muscle. *Physiol. Rev.*, 1936, 16: 450-487.
- GASSER, H. S. Nerve activity as modified by temperature. *Amer. J. Physiol.*, 1931, 97: 254-270.
- GASSER, H. S. The control of excitation in the nervous system. *Harv. Lect.*, 1937, 32: 169-193.
- GASSER, H. S., and GRUNDFEST, H. Action and excitability in mammalian A fibers. *Amer. J. Physiol.*, 1936, 117: 113-133.
- GERARD, R. W. Further observations on oxygen consumption of nerve. *Proc. Soc. exp. Biol.*, N. Y., 1930, 27: 1052-1055.
- GERARD, R. W. Nerve metabolism. *Physiol. Rev.*, 1932, 12: 469-492.
- GERARD, R. W. Factors controlling brain potentials. *Cold Spr. Harb. Symp. quant. Biol.*, 1936, 4: 292-304.
- GERARD, R. W. The metabolism of brain and nerve. *Ann. Rev. Biochem.*, 1937, 6: 419-444.
- GERARD, R. W., and MAGOUN, H. W. Influence of potassium and calcium on motor discharges. *Proc. Soc. exp. Biol.*, N. Y., 1936, 34: 755-756.
- GERARD, R. W., MARSHALL, W. H., and SAUL, L. J. Electrical activity of the cat's brain. *Arch. Neurol. Psychiat.*, Chicago, 1936, 36: 675-738.
- GERARD, R. W., and YOUNG, J. Z. Electrical activity of the central nervous system of the frog. *Proc. roy. Soc.*, 1937, B, 122: 343-352.
- GRAHAM, H. T. Modification of nerve after potential and refractory period by changes in ionic environment. *Amer. J. Physiol.*, 1933, 104: 216-233.
- HEINBECKER, P. The potential analysis of pacemaker mechanism in *Limulus polyphemus*. *Amer. J. Physiol.*, 1936, 117: 686-700.
- HOAGLAND, H. Some pacemaker aspects of rhythmic activity in the nervous system. *Cold Spr. Harb. Symp. quant. Biol.*, 1936, 4: 267-284.
- HOAGLAND, H., RUBIN, M. A., and CAMERON, D. E. Electroencephalogram during insulin hypoglycemia. *Amer. J. Physiol.*, 1937, 120: 559-570.
- HUGHES, J., and GASSER, H. S. The response of the spinal cord to two afferent volleys. *Amer. J. Physiol.*, 1934, 108: 307-321.
- JASPER, H. H. Cortical excitatory state and synchronism in the control of bioelectric autonomous rhythms. *Cold Spr. Harb. Symp. quant. Biol.*, 1936, 4: 320-339.
- LEHMANN, J. E. The effect of changes in pH on the action of mammalian A nerve fibers. *Amer. J. Physiol.*, 1937a, 118: 600-613.
- LEHMANN, J. E. The effect of changes in the potassium-calcium balance on the action of mammalian A nerve fibers. *Amer. J. Physiol.*, 1937b, 118: 613-619.
- LENNOX, W. G., GIBBS, F. A., and GIBBS, E. L. Relationship in man of cerebral activity to blood flow and to blood constituents. *Res. Publ. Ass. nerv. ment. Dis.*, 1938, 18: 277-297.
- LIBET, B., and GERARD, R. W. Automaticity of central neurones after nicotine block of synapses. *Proc. Soc. exp. Biol.*, N. Y., 1938, 38: 886-888.
- LORENTE DE NÓ, R. Analysis of the activity of the chains of internuncial neurones. *J. Neurophysiol.*, 1938, 1: 207-244.
- PROSSER, C. L. Action potentials in the nervous system of crayfish. *J. cell. comp. Physiol.*, 1933, 4: 185-209.
- ROHDE, K. Zur Physiologie der Aufnahme und Ausscheidung saurer und basischer Farbsalze durch die Nieren. *Pflüg. Arch. ges. Physiol.*, 1920, 182: 114-132.
- SCHWEITZER, A., and WRIGHT, S. Action of nicotine on the spinal cord. *J. Physiol.*, 1938, 94: 136-147.
- STARLING, E. H. *Principles of human physiology*. Philadelphia, Lea and Febiger, 1936. xiii., 1096 pp.

